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(באנגלית)
(English)

Polypeptides, DNA molecules encoding them and pharmaceutical compositions

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Signature of Applicant

For the Applicants,
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By: —

היום This 26 בחודש of August שנת 1998 of the year

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פוליפפטידים, מולקולות דנ"א המקודדות להם ותכשירי רוקחות

Polypeptides, DNA molecules encoding them and pharmaceutical compositions

Yeda Research and Development Company Ltd.

The inventors: Rivka DIKSTEIN
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ידע חברה למחקר ופיתוח בע"מ

הממציאים: רבקה דיקשטין
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POLYPEPTIDES, DNA MOLECULES ENCODING THEM AND PHARMACEUTICAL COMPOSITIONS

FIELD OF THE INVENTION

This invention relates to novel fragments of the transcription factor subunit $TAF_{II}105$ and to DNA molecules encoding them, and to pharmaceutical compositions and therapeutic methods using $TAF_{II}105$ and fragments thereof.

BACKGROUND OF THE INVENTION

References referred to in the present specification are listed at the end of the specification before the claims.

Initiation of transcription is a key regulatory step affecting gene expression in response to a variety of extra- and intracellular signals, during developmental processes and for providing tissue specificity. The rate of transcription initiation is determined by proximal and distal enhancer elements that are bound by gene-specific transcription factors (activators or repressors); these are modular in their nature, typically consisting of a DNA binding domain and one or more activation (or repression) domains. The transcription initiation site is determined by a number of general transcription factors (GTFs) that assemble around the core promoter to form the preinitiation complex. The general transcription factor TFIID plays an essential role in transcription initiation, as it recognizes and binds the core promoter and nucleates the assembly of the other general initiation factors

(TFIIA, TFIIB, TFIIE, TFIIF, and TFIIH) and RNA polymerase II (for recent reviews see Hoffman et. al., 1997 and Orphanides *et al.*, 1996).

Studies of transcriptional activation mechanisms revealed that TFIID is also required for mediating transcription-activation signals by gene-specific activators (Horikoshi *et al.*, 1988; Pugh and Tjian, 1990). TFIID is a multisubunit complex that consists of the TATA box- binding protein (TBP) and a number of TBP-associated factors (TAFs) that are present in all cells (Verrijzer and Tjian, 1996). Some of these TAFs have been shown to directly bind activation domains of activators. This interaction appears to be essential for activator-dependent transcriptional stimulation *in vitro* as revealed in reconstituted transcription reactions.

A novel TFIID subunit, $TAF_{II}105$, that is highly enriched in TFIID of human and mouse B-lymphocytes, has recently been identified and cloned (Dikstein *et al.*, 1996, and U.S. 5,710,025, whose contents are incorporated herein by reference). $TAF_{II}105$ exists in sub-stoichiometric amounts relative to the core TAFs, consistent with the assumption that a TFIID complex containing this subunit might be required for transcription of a limited set of genes. $TAF_{II}105$ contains two distinct functional domains: a conserved C-terminal domain implicated in TFIID binding and TAF-TAF interactions (putative amino acids 553-802), and an N-terminal coactivator domain (amino acid 1-552) that directs interaction with activation domains of transcription factors. These findings suggest that $TAF_{II}105$ may be a coactivator that mediates activation signals of gene specific activators.

Apoptosis (or programmed cell death) is a natural cellular mechanism for eliminating unwanted cells in an organism. Apoptosis may occur as a result of an external signal, such as the presence of a cytokine, or in response to an internal cellular signal. Pathological conditions involving apoptosis may occur in several variations. On the one hand, undesired

apoptosis may occur in healthy cells, while on the other hand, apoptosis may be inhibited in diseased cells, such as cancer cells.

The NF-κB family of transcription factors are important regulators of a large number of biological processes. For example, NF-κB plays an essential role in a variety of immune and inflammatory responses, mediates antigen and cytokine-induced gene expression and is required for cellular response to various external signals. The cytokine TNF-α activates NF-κB by inducing rapid nuclear translocation of NF-kB proteins. Recent studies have revealed that NF-κB protects cells from TNF-α induced apoptosis, presumably by activating anti-apoptotic genes (Beg and Baltimore, 1996; Wang et al., 1996; van Antwerp et al., 1996; Liu et al., 1996). Moreover, activation of anti-apoptotic genes by NF-κB has been shown to be essential for transformation by oncogenic Ras (Finco et al., 1997; Mayo et al., 1997). Likewise, activation of NF-κB by ionizing radiation or daunorubicin was found to protect cells from killing by apoptosis (Wang et al., 1996).

SUMMARY OF THE INVENTION

The present invention is based on the surprising discovery that $TAF_{II}105$ interacts directly with members of the NF-κB family and mediates gene activation with respect to apoptotic cellular mechanisms. Although $TAF_{II}105$ was previously described, and it was suggested that $TAF_{II}105$ may be responsible for mediating transcription by a subset of activators in B cells, no indication was given that $TAF_{II}105$ might be involved with NF-κB proteins, which function also in non-B cells, nor with the activation of anti-apoptotic genes.

It is an object of the present invention to provide novel polypeptide fragments of the TFIID polypeptide subunit, $TAF_{II}105$, as well as DNA molecules encoding them.

It is a further object of the invention to provide pharmaceutical compositions and methods for use within the framework of a therapy intended to eliminate undesired, pathological cells, e.g. within the framework of cancer therapy.

It is a still further object of the invention to provide pharmaceutical compositions and methods for treating diseases resulting from the pathological occurrence of apoptosis.

In the present specification, the term "*normal biological activity*" of the $TAF_{II}105$ polypeptide refers to mediation by $TAF_{II}105$ of the activation of anti-apoptotic genes by NF-κB, as described hereinafter.

According to one aspect of the present invention, there is provided a DNA molecule comprising a nucleotide sequence encoding a fragment of the $TAF_{II}105$ polypeptide of SEQ. ID. NO: 2, wherein the fragment has a dominant negative effect on the normal biological activity of the $TAF_{II}105$ polypeptide.

Such fragments may be derived from the N- or C-terminus of the $TAF_{II}105$ polypeptide of SEQ. ID. NO: 2, but preferably they are derived from the N-terminal domain, which is the putative coactivator domain of $TAF_{II}105$. A most preferred fragment consists of the N-terminal amino acids 1 to 552 of $TAF_{II}105$, named herein $TAF_{II}105\Delta C$. Other preferred fragments contain a sequence of amino acids included within $TAF_{II}105\Delta C$ such as the fragments consisting of the amino acids 1-452, 1-359 and 443-452, or is a modified fragment obtained by internal deletion, replacement or addition of one or more amino acids such that the thus obtained modified fragment has a dominant negative effect on the normal biological activity of the $TAF_{II}105$ polypeptide of SEQ. ID. NO: 2.

Based on the discovery, in accordance with the invention, of the role that $TAF_{II}105$ plays in the apoptotic process, the present invention provides novel therapies allowing, on the one hand, to promote apoptosis of pathological cells and, on the other hand, to prevent undesired apoptosis in

normal cells. In the first case, the apoptosis-inhibiting pathway which is mediated by $TAF_{II}105$ is inhibited by neutralizing, directly or indirectly, the $TAF_{II}105$ polypeptide, and in the second case, this apoptosis-inhibiting pathway is enhanced.

In the first aspect of the invention, referred to at times as the "*apoptosis-promoting aspect*", the active $TAF_{II}105$ fragments of the invention are used to promote and potentiate the apoptotic mechanism, for example in the treatment of cancer, in which case it is desired to stimulate apoptosis in the cancerous cell. Thus, the active $TAF_{II}105$ fragment or a composition containing it may be administered by itself to the patient, or it may be administered together with another chemotherapeutic agent so as to increase its effectiveness.

Thus, according to this aspect of the invention, there is provided a pharmaceutical composition for inducing an apoptotic process in pathological cells, for example in the treatment of cancer, comprising a pharmaceutically acceptable carrier and an active agent selected from the group consisting of:

(a) a fragment of the TAF_I105 polypeptide of SEQ. ID. NO: 2 or such a fragment modified by internal deletion, replacement or addition of one or more amino acids, wherein the fragment or the modified fragment has a dominant negative effect on the normal biological activity of the $TAF_{II}105$ polypeptide;

(b) an inhibitor or antagonist of the $TAF_{II}105$ polypeptide of SEQ. ID. NO: 2;

(c) a DNA sequence encoding the fragment of (a); and

(d) a DNA sequence directing expression of an antisense RNA sequence to SEQ.ID. NO.: 1, or a part thereof, and which is capable of inhibiting its expression *in vivo*.

Also provided according to this aspect of the invention is the use of an active agent as defined in **(a) – (d)** above for the preparation of a

pharmaceutical composition for use for promoting apoptosis, for example in the treatment of cancer, as well as a method for the promotion of apoptosis, for example in the treatment of cancer, comprising administering to a subject in need, an active agent as defined in (a) – (d) above or a composition comprising such agent.

In the second aspect of the invention, referred to at times as the "*apoptosis-preventing aspect*", the active agents are used to interfere with the apoptosis mechanism in situations where it is detrimental to the health of the organism. Such situations include, for example, the undesired induction of apoptosis in autoimmune diseases, inflammatory processes and viral or bacterial infections.

Thus, according to this aspect, the present invention provides a pharmaceutical composition for use in the treatment of pathological apoptosis of cells, for example in autoimmune diseases, inflammatory processes and viral or bacterial infections, comprising a pharmaceutically acceptable carrier and an active agent selected from the group consisting of:

(e) a DNA molecule comprising the DNA sequence of SEQ. ID. NO: 1;

(f) a DNA molecule consisting of a DNA sequence encoding the $TAF_{II}105$ polypeptide of SEQ. ID. NO: 2;

(g) a modified DNA sequence of (a) or (b) in which one or more nucleotide triplets have been added, deleted or replaced, wherein the polypeptide encoded by the modified DNA sequence retains the normal biological activity of the $TAF_{II}105$ polypeptide of SEQ. ID. NO: 2;

(h) the $TAF_{II}105$ polypeptide of SEQ. ID. NO: 2; and

(i) a modified polypeptide of (d) in which one or more amino acids have been added, deleted or replaced, wherein the modified polypeptide retains the normal biological activity of the $TAF_{II}105$ polypeptide encoded by SEQ. ID. NO: 2.

Also provided according to this aspect of the invention is the use of an active agent as defined in (e) – (i) above for the preparation of a pharmaceutical composition for use in the treatment of pathological apoptosis of cells, for example in autoimmune diseases, inflammatory processes and viral or bacterial infections, as well as a method for the treatment of pathological apoptosis of cells, for example in autoimmune diseases, inflammatory processes and viral or bacterial infections,, comprising administering to a subject in need, an active agent as defined in (e) – (i) above or a composition comprising such agent.

The aforementioned active agents of both aspects of the invention may be administered to an individual by the use of appropriate carriers, which may be selected from a number of such carriers known *per se*. For example, where said active agent is a DNA sequence or a modified DNA sequence, it will typically be administered within the framework of a vector, as known in the art of gene therapy. As will be appreciated, the vector, which may be a plasmid, viral particle, etc., should preferably be constructed so that it will direct the active DNA sequence into the target cells, namely, cells in which apoptosis is to be induced or cells undergoing undesired apoptosis. Furthermore, the vector should also have the necessary promoters so as to induce expression of the DNA sequence. The manner of construction of appropriate vectors including ensuring appropriate targeting and expression control of the inserted DNA sequence, are generally known and the man of the art should have no difficulties, based on the teaching of the present invention and the general knowledge available in the art, to construct the appropriate vectors.

It is obviously also within the reach of the artisan to prepare pharmaceutical compositions where the active agent is a peptide or polypeptide. At times, it may be desired to include a targeting vehicle in the composition so as to target the polypeptide to the cells, which vehicle may, for

example, be a liposome having a targeting moiety, e.g. a ligand of the receptor, on its external surface.

As is also known, the targeting of the inventive compositions to the required site within the body, may also be achieved by direct injection or infusion of the compositions into the site.

DETAILED DESCRIPTION OF THE DRAWINGS:

In order to understand the invention and to see how it may be carried out in practice, preferred embodiments will now be described, by way of non-limiting example only, with reference to the accompanying drawings, in which:

Fig. 1 is the DNA sequence encoding the $TAF_{II}105$ polypeptide (SEQ. ID. NO: 1);

Fig. 2 is the deduced amino acid sequence of the $TAF_{II}105$ polypeptide (SEQ. ID. NO: 2);

Figs. 3A-3C illustrate the interaction of $hTAF_{II}105$ with p65/RelA, a member of the NF-κB family.

3A. NF-κB members p65/RelA and p50 and unrelated OCT2 proteins were translated *in vitro* and labeled with ^{35}S-methionine using rabbit reticulocyte lysate. These proteins were used for interaction assay with immobilized flag-tagged $TAF_{II}105$ as indicated on the top of each lane. As a control, the labeled proteins were incubated with the flag beads. The bound proteins were eluted, resolved on SDS-PAGE and autoradiographed. Input lanes represent 10% of the labeled protein used for the binding reaction. **3B.** Binding reaction between ^{35}S-labeled p65/RelA and the purified N-terminal fragment of $TAF_{II}105$ (amino acids 1-552) fused to glutathione S-transferase (GST) and bound to glutathione beads (lane 2). As a control, a similar reaction was performed using the same beads bound by GST (lane 3). Input lanes represent 10% of p65 used for the binding assay. **3C.** Interaction between $TAF_{II}105$ and the C-terminal activation domain of

p65. The C-terminus of p65 (amino acids 398-551) was expressed as a GST- fusion protein, affinity-purified by glutathione beads and subjected to a binding reaction with *in vitro* translated and ^{35}S-labeled TAF$_{II}$105 (lanes 1-3) or TAF$_{II}$130 (lanes 4-6). GST-containing beads were used as control in a similar binding reaction. Input lanes represent approximately 10% of the labeled proteins used.

Figs. 4A & 4B illustrate the interaction of additional NF-κB rel proteins with TAF$_{II}$105.

4A. *In vitro* translated and ^{35}S-labeled RelB (lanes 1-3) and c-rel (lanes 4-6) were used for an *in vitro* binding assay with the N-terminus of TAF$_{II}$105 similar to Fig. 3 B. **4B.** The C-termini of human c-rel (amino acids 302-614) and mouse relB (amino acids 348-558) were expressed as fusion with GST protein, purified by glutathione beads and used for binding assay with *in vitro* translated and ^{35}S-labeled TAF$_{II}$105 (lanes 1-4) or TAF$_{II}$130 (lanes 5-8). Input lanes represent 10% of the labeled proteins used in the assay.

Figs. 5A-5D illustrate the stimulation of p65 and TNF-α induced NF-κB transcriptional activity by TAF$_{II}$105.

5A. Human 293A cells (in 24-well plate) were cotransfected with NF-κB-dependent luciferase reporter plasmid (50 ng) together with either an empty expression vector or the following expression plasmids: 500 ng of TAF$_{II}$105 (columns 2, 4, 7, 8 and 10); 12.5 ng of p65 (columns 5 and 7); 25 ng of p65 (columns 6 and 8) and 500 ng of IkBα (columns 3, 4, 9 and 10). The amount of CMV-derived vector in each transfection assay was kept constant. **5B.** Similar transfection experiment using HeLa cells. In this experiment the amount of DNA used was 10-fold higher, keeping the relative amount of each plasmid similar. **5C.** An NF-κB luciferase reporter plasmid was cotransfected into 293A cells using similar transfectioconditions as in A, with either an empty expression vector (columns 1, 3, 7), a TAF$_{II}$105 expression plasmid (columns 2,5,6, and 8) or

with a constitutively active IkBα(lanes 7 and 8). 12 hours post-transfection, 0.15 ng/ml (columns 3 and 4) or 1.5 ng/ml (columns 5-8) of TNF-α were provided to the cells, and 24 hours after transfection, luciferase activity was determined. **D.** Similar transfection experiment in HeLa cells using 0.15 ng/ml TNF-α.

Figs. 6A-6C illustrate the inhibition of NF-κB transcription by dominant negative mutant of $TAF_{II}105$ ($TAF_{II}105\Delta C$).

6A. Schematic representation of $TAF_{II}105$ putative functional domains and the dominant negative mutant of $TAF_{II}105$ ($TAF_{II}105\Delta C$). NLS stands for nuclear localization signal. Expression of mutated $TAF_{II}105$ was confirmed by Western blot (data not shown). **6B.** Luciferase reporter plasmids driven by two tandem NF-κB sites (lanes 1-3), two mutated NF-κB sites (lanes 4 and 5) or a CMV enhancer (lanes 6 and 7) were transfected into 293A cells together with the following plasmids: empty expression vector (lanes 1, 4 and 6), with $TAF_{II}105\Delta C$ expression plasmid (lanes 2, 5 and 7) or dominant negative mutant of IkBα (lane 3). **6C.** An NF-κB reporter plasmid (50 ng) was transfected into 293A cells together with an empty expression vector (lane 1), 25 ng of p65/RelA expression plasmid (lanes 2-4) and increasing amounts of $TAF_{II}105\Delta C$ expression plasmid (100 and 250 ng, lanes 3 and 4, respectively).

Figs. 7A-7C illustrate how a dominant negative mutant of $TAF_{II}105$ induces cell death in response to TNF-α.

7A. 293T cells were transfected with wild-type $TAF_{II}105$ (pictures 1 and 2) or $TAF_{II}105\Delta C$ (3 and 4), and either untreated (1 and 3) or treated with 15 ng/ml TNF-α (2 and 4). The pictures were taken 24 hours after transfection. **7B.** Genomic DNA analysis of 293T cells that were transfected with either an empty expression vector (lanes 1 and 2), $TAF_{II}105\Delta C$ (lanes 3 and 4) or with wild-type $TAF_{II}105$ (lanes 5 and 6). Twelve hours after transfection some of the transfected cells were treated with TNF-α (odd number lanes), and 24 hours after transfection DNA was

extracted from the transfected cells and analyzed by 1.8% agarose gel. M stands for a 1 kB DNA size marker. **7C.** Cell survival assay. To quantify the apoptosis induction, 293T cells were cotransfected with a pCMV*lacZ* reporter plasmid and the indicated expression vectors, and similar to the experiment described in (B), were treated with TNF-α (lanes 2, 4 and 6). 24 hours after transfection the cells were stained with X-Gal, and the numbers of blue cells in five randomly chosen fields were determined. These data represent an average of 3 independent transfection experiments.

Figs. 8A-8C illustrate the effect of $TAF_{II}105$ anti-sense RNA expression on TNF-α treated cells.

8A. 293T cells were transfected either with empty vector or with expression vector for $TAF_{II}105$ anti-sense RNA. Thirty-six hours after transfection the levels of endogenous $TAF_{II}105$ were analyzed by Western blot using affinity-purified anti-$TAF_{II}105$ antibodies (upper panel). As a control the same extracts were used for analysis of TBP levels (lower panel). **8B.** Cell-survival analysis of cells expressing $TAF_{II}105$ anti-sense RNA. 293T cells were cotransfected with CMV-GFP reporter plasmid with either an empty expression vector (lanes 1 and 2) or with expression vector for $TAF_{II}105$ anti-sense RNA (lanes 3 and 4). Twenty-four hours after transfection TNF-α was provided to the cells (lanes 2 and 4) and 24 hours later green fluorescent cells were counted in five randomly chosen fields. These data represent an average of four independent transfection experiments. **8C.** DNA fragmentation analysis of cells transfected with either anti-sense $TAF_{II}105$ RNA (lanes 1 and 2) or empty vector (lanes 3 and 4). Twenty-four hours after transfection TNF-α was provided to the cells (lanes 1 and 3) and 16 hours later fresh medium containing TNF-α was provided to the cells. Genomic DNA was extracted 48 hours after transfection and analyzed by 1.8% agarose gel. M represents a 1 kB DNA molecular size marker.

Figs. 9A-9C illustrate how a dominant negative mutant of $TAF_{II}105$ inhibits activation of anti-apoptotic genes by NF-κB.

9A. 293T cells were transfected with the indicated expression plasmids. 24-hours after transfection, DNA was extracted from the cells and analyzed by 1.8% agarose gel. **9B.** A representative experiment as described in A. The pictures were taken 24 hours after transfection. 293T cells transfected with p65 are shown in panel 1; cells cotransfected with wild-type $TAF_{II}105$ and p65 are shown in panel 2; and cells cotransfected with $TAF_{II}105\Delta C$ and p65 are shown in panel 3. Pictures of cells transfected with either $TAF_{II}105$ or $TAF_{II}105\Delta C$ alone are shown in Fig. 7. **9C.** 293T cells were cotransfected with pCMV*lacZ* reporter plasmid and expression vectors as indicated in the figure. Twenty-four hours after transfection the cells were stained with X-Gal. The numbers of blue cells in five randomly chosen fields were determined. These data represent an average of 3 independent transfection experiments.

Figs. 10A & 10B schematically illustrate a model for the role of $TAF_{II}105$ (TFIID) in activation of TNF-α/NF-κB induced anti-apoptotic genes in 293 cells;.

Fig. 11 is a schematic representation of various deletion mutants of the $TAF_{II}105$ N-terminal domain: amino acid 1-552, 1-452, 1-359, 1-167 and 443-452, and their ability to bind p65 and their effect on the survival of p65 expressing cells.

Fig. 12A shows the structure of a $TAF_{II}105\Delta C$ transgene. The DNA used to generate $TAF_{II}105\Delta C$ transgenic animals contains a cDNA fragment encoding for the N-terminus (amino acid 1-552) of $TAF_{II}105$, an in frame nuclear localization signal (NLS) and influenza virus hemagglutinin tag (HA). **12B.** Southern blot showing the integration of the transgene into the genome of mice. Restriction enzyme-digested tail DNA of several founders was analyzed for the integration of $TAF_{II}105\Delta C$ by Southern blot using $TAF_{II}105\Delta C$ as a probe. PC is the positive control.

DETAILED DESCRIPTION OF THE INVENTION

MATERIALS AND METHODS

$TAF_{II}105$

$TAF_{II}105$ was prepared as previously described (Dikstein *et al.*, 1996). The DNA and amino acid sequences are shown in Figs. 1 and 2, respectively.

In vitro binding experiments

Baculovirus expression of flag-tagged $TAF_{II}105$ was carried out as previously described (Dikstein *et al.*, 1996). ^{35}S-labeled p65, OCT2, and p50 were synthesized *in vitro* by T7 RNA polymerase and rabbit reticulocytes lysate and incubated with flag beads or with $TAF_{II}105$ coupled to flag beads in 0.1M KCl HEMG buffer (20 mM Hepes pH 7.9, 100 mM KCl, 12.5 mM $MgCl_2$, 0.2 mM EDTA, 0.1% NP-40, 1mM DTT, 0.2mM PMSF) for 2 hours at 4°C. The beads were washed 3 times with the same buffer and 2 times with 0.2M NaCl HEMG buffer. The bound proteins were eluted by 5 minutes boiling in protein sample buffer followed by SDS-PAGE and autoradiography. The c-rel protein was translated *in vitro* using T7 RNA polymerase. RelB was synthesized by T3 RNA polymerase. Binding reactions with GST-bound proteins were done in a similar way.

Plasmids

The baculovirus expression vector for $TAF_{II}105$ was previously described (Dikstein *et al.*, 1996). CMV-$TAF_{II}105$ was constructed by inserting a NcoI-BglII (NcoI was filled in by the Klenow enzyme) fragment containing $TAF_{II}105$ cDNA and HA Tag from pVLHA- $TAF_{II}105$ (Dikstein *et al.*, 1996) into SmaI-BamHI site of the pCGN vector (Tanaka and Herr, 1990).

To generate the $TAF_{II}105\Delta C$ expression vector, an N-terminal fragment of $TAF_{II}105$ (amino acid 1-552, Dikstein *et al.*, 1996) that was

generated by PCR together with an HA tag was first cloned in a CMV-nuc vector (a gift from Dr. Paz Einat) in frame with a nuclear localization signal (NLS). Next, a PstI-HindIII fragment containing NLS, HA and $TAF_{II}105\Delta C$ was inserted into PstI-HindIII sites BlueScript KS+ vector. This fragment was then removed by SmaI and HincII and cloned in a SmaI site of pCGN. The vector for directing anti-sense $TAF_{II}105$ RNA expression contains the same insert, albeit in opposite orientation. Expression of both wildtype and mutant $TAF_{II}105$ after transient transfecwas verified by Western blot using anti-HA antibodies (data not shown).

GST- $TAF_{II}105\Delta C$ was generated by cloning an NdeI-EcoRI fragment from pET- $TAF_{II}105\Delta C$ (Dikstein *et al.*, 1996) into NdeI-EcoRI of pGEX-2TKN. The pTβSTOP-c-rel that was used for *in vitro* translation of c-rel protein was constructed by insertion of an NcoI-EcoRI fragment containing c-rel cDNA (a gift from Dr. Nancy Rice) into NcoI-EcoRI sites of the pTβSTOP vector. GST-p65C was constructed by inserting an NcoI-XhoI fragment (corresponding to amino acids 398-551) from pET-p65 into NcoI-SalI of pGEX-2TKN. To generate GST-c-relC, a fragment corresponding to amino acids 302-614 was cloned into a StuI site of pGEX2TKN. GST-RelBC was generated by inserting a BglII-BamHI fragment from pBS-RelB (amino acids 348-558, a gift from Dr. Rodrigo Bravo) into a BamHI site of pGEX-2TKN.

The NF-κB dependent reporter plasmids (wild-type and mutant) were constructed by inserting double-stranded synthetic oligonucleotides (see below) containing either two tandem κB sites or two mutated κB sites next to a minimal core promoter from the mouse α-actin promoter (-40 to +80), that was cloned into Hind III site of a promotorless pLuc vector (Altschmied and Duschl 1997).

NF-κB oligonucleotides:

5'-AGCTTAGGGACTTTCCGAGGGGACTTTCCG-3';

5'GATCCGGAAAGTCCCCTCGGAAAGTCCCTA-3'

Mutated NF-κB oligonucleotides:

5'AGCTTATCTACTTTCCGAGTCTACTTTCCG-3';

5'-GATCCGGAAAGTAGACTCGGAAAGTAGATA-3'.

Propagation and Transfection of Cell Lines

293 and HeLa cell lines were maintained in F12 Dulbecco's modified Eagles medium (DMEM) supplemented with 10% fetal calf serum. Transfections were performed using the standard $CaPO_4$ method.

293 subconfluent cells were transfected in a 24-well multidish using a total of 1 μg plasmid; HeLa cells were transfected using 10 μg of plasmid/$5X10^6$ cells in 100mm petri dish. Luciferase activity was determined according to the instructions of the manufacturer (Promega).

Human recombinant TNF-α was purchased from R & D Systems.

Apoptosis assays

For the survival analysis, 293A or 293T cells were cotransfected with a pCMV-*lacZ* reporter plasmid and different expression vectors and stained with X-Gal (see below) 36 hr or 48 hr after transfection. The number of blue cells was determined by counting five different randomly chosen fields.

For the X-Gal assay the cultures were rinsed with PBS, fixed with 1% glutaraldehyde for 15 min, and then stained with 4 ml of X-Gal solution [2 mg/ml X-Gal in DMF, 3.3 mM $K_3Fe(CN)_6$ and 3.3 mM $K_4Fe(CN)_6$] for 2 hr.

DNA fragmentation assay was carried out as follows: 293T cells ($2X10^6$) were collected 24 hr after transfection, resuspended in 400μl lysis buffer (200 mM Tris pH 8.5, 100 mM EDTA, 1% SDS, and 100 μg/ml proteinase K) and incubated overnight at 37 [illegible] phenol extraction following ethanol precipitation. Pelleted DNA was

resuspended in 100 µl TE containing 50 µg/ml RNase A and incubated for 30 min at 37°C. Twenty micrograms of DNA was run on a 1.8% agarose gel in the presence of 0.5 µg/ml ethidium bromide.

EXAMPLES

I. $TAF_{II}105$ interacts with the trans-activation domains of NF-κB members

To identify $TAF_{II}105$ target activators, the transcription factors NF-κB and OCT2 that are involved in B cell specific transcription were tested as to whether they could interact with $TAF_{II}105$. For this purpose, recombinant $TAF_{II}105$ was produced in Sf9 cells as a fusion with the flag epitope-tag and purified by anti-flag antibodies coupled to agarose beads. Immobilized $TAF_{II}105$ was incubated with *in vitro* translated and ^{35}S-labeled p50 and p65 (relA) subunits NF-κB family and OCT2.

As shown in Fig. 3A, p65 but not p50 or OCT2 specifically and efficiently interacts with $TAF_{II}105$. To determine whether the interaction between p65 and $TAF_{II}105$ is directed by the putative N-terminal coactivator domain of $TAF_{II}105$, this region was expressed in *E. Coli* as a fusion with glutathione S-transferase (GST105ΔC) and subjected to a binding reaction with ^{35}S-labeled p65. As shown in Fig. 3B, p65 specifically binds to the N-terminus of $TAF_{II}105$. We also tested other members of the NF-κB family (c-rel and RelB) for interaction with $TAF_{II}105$. c-rel but not RelB binds to $TAF_{II}105N$ in this assay (Fig. 4A).

Previous studies of activator-TAF association established that an interaction between coactivator subunits of TFIID and transcriptional activators is directed by the trans-activation domain of the activator (Verrijzer and Tjian, 1996). Both p65 and c-rel contain a strong activation domain located at the carboxy-terminus (reviewed by Schmitz and Baeuerle, 1995). To determine the involvement of these activation domains in $TAF_{II}105$ binding, we constructed plasmids for expression of the p65, c-rel

and RelB C-termini as fusion with glutathione S-transferase (GST-p65C, GST-RelBC and GST-c-relC). These proteins were purified and immobilized on glutathione Sepharose beads and used in binding assays with an *in vitro* translated and ^{35}S-labeled TAF$_{II}$105. To determine the specificity of the interaction, a similar binding reaction was also performed with h TAF$_{II}$130, a highly related homologue of TAF$_{II}$105 (Dikstein *et al.*, 1996). The C-terminal activation domains of both p65 and c-rel specifically bind TAF$_{II}$105 but not TAF$_{II}$130 (Figs. 3C and 4B). In this assay, the activation domain of RelB weakly interacts with TAF$_{II}$105 (Fig. 4B).

II. NF-κB dependent transcription is stimulated by TAF$_{II}$105

To determine the functional importance of TAF$_{II}$105-NF-κB interaction, we tested the effect of TAF$_{II}$105 on transcriptional activation by p65 in transient transfection experiments. A reporter plasmid containing two NF-κB elements upstream to a minimal core promoter and a luciferase gene was cotransfected with p65 and TAF$_{II}$105 into 293 cells. As shown in Fig. 5A, p65 activates NF-κB reporter in a dose-dependent manner (columns 5 and 6). When TAF$_{II}$105 was also cotransfected, both the basal NF-κB and p65 dependent activities were stimulated 2, 5 and 6 fold respectively (columns 2, 7 and 8). Likewise, TAF$_{II}$105 potentiates NF-κB dependent transcription in HeLa cells (Fig. 5B).

To determine whether the activation by TAF$_{II}$105 requires the presence of NF-κB proteins, a similar experiment was carried out in the presence of IκBα, a specific inhibitor of NF-κB factors that prevents the translocation of NF-κB into the nucleus (reviewed in Verma *et al.*, 1995; Baldwin, 1996; Baeuerle and Baltimore, 1996). As expected, IκBα inhibits both constitutive and p65 induced NF-κB activity (Fig. 5A, columns 3 and 9 respectively). In the presence of IκB, no induction of luciferase activity by TAF$_{II}$105 is observed (Fig. 5A, columns 4 and 10).

NF-κB activity is regulated by a broad range of cytokines and external stimuli. TNF-α is among the physiological inducers of NF-κB transcriptional activation. To test whether TAF$_{II}$105 can stimulate TNF-α induced genes, the NF-kB reporter plasmid was transfected into 293 and HeLa cells in the presence or absence of a TAF$_{II}$105 expression plasmid. When the cells are stimulated with sub-optimal doses of TNF-α (0.15 and 1.5 ng/ml), TAF$_{II}$105 stimulates the NF-κB dependent reporter in response to TNF-α (Figs. 5C and 5D). Here again, in the presence of IkB no induction of NF-kB reporter by TAF$_{II}$105 is observed (Fig. 5C, columns 7 and 8). Interestingly, when optimal doses of TNF-α are provided to the cells (10-15 ng/ml), or when the cells are transfected with high amount of p65 expression plasmid, cotransfected TAF$_{II}$105 has little or no additional effect on the NF-κB dependent reporter plasmid that is stimulated to its maximal potential (data not shown) possibly because the basal level of TAF$_{II}$105 present in the cells is sufficient for transcriptional activation when NF-κB concentration within the nucleus is high. These experiments are representative of 3-5 independent transfection experiments with similar results. Expression of transfected TAF$_{II}$105 was verified by Western blot (data not shown).

To further confirm that TAF$_{II}$105 is involved in NF-κB activity a eukaryotic expression vector for a dominant negative mutant of TAF$_{II}$105 was constructed based on its predicted functiodomains (Dikstein *et al.*, 1996). This mutant is a truncated form of h TAF$_{II}$105 (TAF$_{II}$105ΔC, amino acids 1-552, Fig. 6A), containing the NF-κB binding region but not the TFIID interaction region. Therefore, if TAF$_{II}$105ΔC binds NF-κB *in vivo*, it is likely to inhibit NF-κB dependent transcription.

To test the effect of TAF$_{II}$105ΔC on NF-κB activity, the NF-κB dependent reporter plasmid was cotransfected with TAF$_{II}$105ΔC into 293 cells. The constitutive basal activity of NF-κB was strongly inhibited by TAF$_{II}$105ΔC (Fig. 6B). Similarly, cotransfection of the NF-κB reporter

with a dominant negative IκB-α inhibited this basal NF-κB activity, confirming that the observed activity is directed by NF-κB proteins (column 3). Reporter plasmids containing either mutation within the NF-κB sites (columns 4 and 5) or the CMV enhancer (columns 6 and 7) were not affected by $TAF_{II}105\Delta C$ further supporting the idea that $TAF_{II}105\Delta C$ is specific to NF-κB and has no effect on core promoter activity.

It was next examined whether a dominant negative mutant of $TAF_{II}105$ can interfere with the trans-activation potential of NF-κB proteins. 293 cells were cotransfected with NF-κB dependent reporter plasmid and p65/RelA transcription factor together with $TAF_{II}105\Delta C$. As shown in Fig. 6C, $TAF_{II}105\Delta C$ inhibited transcriptional activation by p65/RelA in a dose dependent manner. It is important to note that the amounts of $TAF_{II}105\Delta C$ expression plasmid used in this experiment are insufficient to affect cell viability when cotransfected with p65 (see Fig. 10 below), as evident by microscopic examination and X-gal staining of transfected cells (data not shown).

III. Inhibition of $TAF_{II}105$ function causes apoptosis in response to TNF-α

Recent studies have revealed that activation of NF-κB by TNF-α plays an essential role in protecting cells from pro-apoptotic stimuli produced by TNF-α (Beg and Baltimore, 1996; Wang *et al.*, 1996; Van Antwerp *et al.*, 1996; Liu *et al.*, 1996). Interestingly, when the effect of the $TAF_{II}105$ dominant negative mutant on long term TNF-α stimulation in 293 cells was analyzed, we observed dramatic changes in 293 cell morphology that are consistent with cell death (Fig. 7A). These morphological changes were not observed in cells transfected with vector alone (data not shown) or with wild type $TAF_{II}105$ (Fig. 7A). The survival rate after TNF-α stimulation in $TAF_{II}105\Delta C$ expressing cells was less than 5% as determined by X-gal staining of transfected cells (Fig. 7C).

To determine whether these changes are related to programmed cell death, 293 cells transiently expressing $TAF_{II}105\Delta C$ were induced by TNF-α and assayed for the DNA ladder characteristic to apoptotic response (Fig. 7B). As expected, no DNA fragmentation appears in TNF-α induced 293 cells transfected with empty expression vector (lane 1). The DNA ladder can be clearly seen only in cells expressing $TAF_{II}105\Delta C$ and stimulated with TNF-α (lane 3), whereas no DNA fragmentation appears in these cells in the absence of TNF-α stimulation (lane 4), or in cells expressing wild type $TAF_{II}105$ in the presence or absence of TNF-α stimulus (lanes 5 and 6).

To further confirm the involvement of $TAF_{II}105$ in activation of anti-apoptotic genes by TNF-α, 293 cells were transiently transfected with expression vector that directs expression of anti-sense $TAF_{II}105$ RNA. As shown in Fig. 8A, transient expression of anti-sense $TAF_{II}105$ reduced the endogenous level of $TAF_{II}105$ protein but not TBP. This reduction is significant considering that although high transfection efficiencies are achieved in these cells, there is still a significant proportion of untransfected or poorly transfected cells that express normal amounts of $TAF_{II}105$ (approximately 20% as determined by X-gal staining after transfection, data not shown). The reduction in $TAF_{II}105$ level is correlated with an increase in cell death in response to TNF-α, as evident by cell survival assay (Fig. 8B), by DNA fragmentation (Fig. 8C) and microscopic examination (data not shown).

IV. $TAF_{II}105$ is involved in activation of anti-apoptotic genes by NF-κB

In addition to the role that NF-κB plays in activation of anti-apoptotic genes, numerous reports implicated a role for NF-κB in activation of pro-apoptotic signals (Lee *et al.*, 1995; Lin *et al.*, 1995; Grimm *et al.*, 1996). In 293 cells the involvement of NF-κB proteins in both

processes has been documented (Wong et al., 1989; Grimm *et al.*, 1996). When 293 cells are transfected with p65, the cells stay healthy and do not die by apoptosis (Figs. 9A, B and C), presumably because of equilibrium between pro- and anti- apoptotic signals produced by p65. Also, cotransfection of $I_\kappa B$ and p65 has no effect on cell survival (Fig. 9A), since $I_\kappa B$ prevents NF-κB from entering the nucleus and therefore inhibits any transcriptional activity by p65. Surprisingly, cotransfection of p65 with the dominant negative mutant of $TAF_{II}105$ resulted in a strong apoptotic response as evident by DNA fragmentation, the characteristic morphological changes, and surviving cell number (Fig. 9A, B and C respectively). By contrast, no effect is observed with cotransfection of p65 and wild type $TAF_{II}105$ (Figs. 9A and B) or by expression of each of these factors alone (Fig. 7). These results confirm the direct role of p65 in activation of cell death in 293 cells and suggest that the $TAF_{II}105$ mutant inhibits activation of anti-apoptotic genes but not pro-apoptotic genes by p65. Therefore, it is likely that $TAF_{II}105$ is required only for activation of anti-apoptotic genes by NF-κB.

A proposal outlining $TAF_{II}105$ activity is summarized in Figs. 10A and 10B. TNF-α activates both anti- and pro-apoptotic cascades (Fig. 10A). The anti-apoptotic pathway requires $I_\kappa B$ phosphorylation and degradation followed by NF-κB nuclear localization. In the nucleus, a complex that is formed between NF-κB and TFIID containing $TAF_{II}105$ selectively promotes transcription of genes that antagonize the protein synthesis-independent cytotoxic pathway induced by TNF-α. Various agents that can induce NF-κB are also known to stimulate apoptotic signals in cells, such as H_2O_2 derived radicals, UV-irradiation, viruses, etc. In 293 cells, activation of NF-κB causes transcriptional activation of both genes that promote apoptosis as well as genes that inhibit it. The NF-κB-$TAF_{II}105$-TFIID complex is selectively engaged in activation of

anti-apoptotic genes by NF-κB, while the mechanism of activation of pro-apoptotic genes by NF-κB remains obscure (Fig. 10B).

V. Preparation of dominant-negative TAF$_{II}$105 deletion mutants

On the basis of the results described above, it is possible to inhibit specific functions of native TAF$_{II}$105 in a dominant negative manner by using small fragments of the TAF$_{II}$105 N-terminus corresponding to different activator binding sites. For example, mapping of NF-κB binding region within TAF$_{II}$105 can provide more efficient and specific inhibitors of NF-κB dependent activation of anti-apoptotic genes. In order to prepare such fragments, it is possible to generate deletion mutants of the TAF$_{II}$105 N-terminus. These mutants may be first expressed in *E. Coli* as fusion proteins such as with glutathione S-transferase, and are then used in *in vitro* binding assays with different TAF$_{II}$105 target activators such as NF-κB members.

To further investigate the involvement of TAF$_{II}$105 in NF-κB induction of anti-apoptotic genes we generated deletion mutants of TAF$_{II}$105ΔC. Various fragments from TAF$_{II}$105 cDNA corresponding to amino acids 1-452, 1-359, 1-167 and 443-552 were generated by restriction enzymes or by PCR. These mutants were expressed in E. Coli, purified and analyzed for their ability to bind p65/RelA in an in vitro binding assay with p65/RelA and for their effect on the survival of p65 expressing cells by expressing the fragments in human 293 cells together with p65.

As shown in Fig. 11, TAF$_{II}$105ΔC (1-552) potently inhibits cell survival (+++) and binds p65 with high affinity (+++). Mutants of TAF$_{II}$105ΔC deleted of 100 or 193 amino acid residues [105 ΔC (1-452) and 105ΔC (1-359)] have reduced p65 binding capacity (+) as well as reduced inhibitory activity (+) as comwith 105ΔC. Similarly, a polypeptide fragment corresponding to amino acid 443-552 is capable of p65 binding and inhibits its anti-apoptotic activity (++) while a fragment (105 ΔC 1-167) that is unable to bind p65 (-) did not affect cell survival (-).

These findings indicate the existence of two independent p65 binding domains within $TAF_{II}105\Delta C$ and strongly suggest that the direct interaction between $TAF_{II}105$ and p65/RelA plays a role in activation of anti-apoptotic genes.

VI. $TAF_{II}105\Delta C$ transgenic mice

Another approach which may be taken to study the *in vivo* function of $TAF_{II}105$ is to generate a transgenic mouse model that expresses dominant negative mutants of $TAF_{II}105\Delta C$. To construct a $TAF_{II}105\Delta C$ transgene, $TAF_{II}105$ cDNA encoding for the non-conserved N-terminus (amino acid 1-552) cloned in pCGN was used. This is a vector that allows high expression level of a transgene. In addition, an in frame nuclear localization signal and influenza virus hemaglutinin tag (HA) were added to allow nuclear transport of the transgene product and easy detection by anti-HA antibody (Fig. 12A).

This DNA was microinjected into fertilized mouse eggs that were recovered from superovulated females that had previously mated with males. At the 2-cell stage the embryos were implanted into the oviducts of pseudopregnant foster animals. Using this procedure, several animals were developed from microinjected eggs. These animals were examined for the transgene by Southern blot analysis using total genomic DNA that was prepared from tail biopsies several weeks after birth. As shown in Fig. 12B, several positive animals in which the transgene has been stably integrated were obtained. The positive animals were mated with wild-type animals. After the birth of the F1 generation, the expression of the transgene mRNA and protein are determined.

To determine whether expression of a dominant negative mutant of $TAF_{II}105$ has an effect on the transgenic animals, and particularly on the composition of the hematopoietic cell population, the health of transgenic animals of different ages may be compared to non-transgenic wild-type animals. In addition, a histological examination may be performed on spleen, thymus, lymph node, bone marrow, liver and if necessary other tissues. Furthermore, thymus and spleen organs may be subjected to immunostaining analysis using antibodies

directed against different types of lymphocytes. Subsequently, a cell suspension prepared from hematopoietic tissues such as spleen, thymus and bone marrow may be subjected to FACS analysis using a panel of differentiation markers, and compared to wild-type animals. Both techniques allow the identification of cell populations which are affected by this mutant.

It is of particular interest to compare the phenotype of these animals with those of NF-κB, and TNF-α ligand and receptor deficiency. If an affected population is found, the mRNA levels of some tissue/stage specific genes may be measured by Northern blot analysis and compared to those of wild-type animals. mRNAs which will be affected by hTAF$_{II}$105ΔC expression are likely to be its target genes (direct or indirect). Also, primary cells (B or T) from the transgenic animals may be cultured and analyzed for normal cellular processes of this particular tissue, such as transcriptional activation of certain promoters, the specific response to a certain cytokine, etc.

If an *in vivo* effect is found, the region within the TAF$_{II}$105 N-terminus which confers this effect upon overexpression may be determined. To this end, new transgenic mice may be generated expressing different mutants of TAF$_{II}$105ΔC according to the *in vitro* binding assays and the transfection results. The phenotype of the transgenic animals may be determined in a similar manner. It is of particular interest to determine if there is a correlation between the activator binding sites and the *in vivo* effect, thus linking the biochemical and genetic approaches.

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CLAIMS:

1. A DNA molecule comprising a nucleotide sequence encoding a fragment of the $TAF_{II}105$ polypeptide of SEQ. ID. NO: 2 or such a modified fragment, wherein said fragment or modified fragment has a dominant negative effect on the normal biological activity of said $TAF_{II}105$ polypeptide.

2. A DNA molecule according to Claim 1 wherein said fragment is derived from the N-terminal domain of the $TAF_{II}105$ polypeptide of SEQ. ID. NO: 2.

3. A DNA molecule according to Claim 2 of SEQ. ID. NO: 1 wherein said nucleotide sequence encodes a $TAF_{II}105\Delta C$ polypeptide consisting of amino acids 1 through 552 of SEQ. ID. NO: 2, or a part thereof.

4. A DNA molecule according to Claim 1 wherein said nucleotide sequence encodes a fragment of the $TAF_{II}105\Delta C$ polypeptide having the amino acid sequence selected from the group consisting of amino acids 1-452, 1-359 and 443-552.

5. A DNA molecule according to Claim 1 wherein said nucleotide sequence encodes a modified fragment obtained by internal deletion, replacement or addition of one or more amino acids of the $TAF_{II}105\Delta C$ polypeptide such that the thus obtained modified fragment has a dominant negative effect on the normal biological activity of the $TAF_{II}105$ polypeptide of SEQ. ID. NO: 2.

6. An expression vector comprising the DNA molecule of any one of Claims 1-5 and DNA sequences required for its expression.

7. A polypeptide consisting of a fragment of the $TAF_{II}105$ polypeptide of SEQ. ID. NO: 2 or a modified fragment thereof, wherein said fragment or modified fragment has a dominant negative effect on the normal biological activity of said $TAF_{II}105$ polypeptide.

8. A polypeptide according to Claim 7 which is a fragment derived from the N-terminal domain of the $TAF_{II}105$ polypeptide of SEQ. ID. NO: 2.

9. A polypeptide according to Claim 8 of SEQ. ID. NO: 1, which is the $TAF_{II}105\Delta C$ polypeptide consisting of amino acids 1 through 552 of SEQ. ID. NO: 2, or a part thereof.

10. A polypeptide according to Claim 9 which is a fragment of the $TAF_{II}105C$ polypeptide selected from the group consisting of amino acids 1-452, 1-359 and 443-552.

11. A polypeptide according to Claim 7 which is a modified fragment obtained by internal deletion, replacement or addition of one or more amino acids of the $TAF_{II}105\Delta C$ polypeptide such that the thus obtained modified fragment has a dominant negative effect on the normal biological activity of the $TAF_{II}105$ polypeptide of SEQ. ID. NO: 2.

12. A DNA molecule directing expression of an antisense RNA sequence to SEQ .ID. NO.: 1, or a part thereof, and which is capable of inhibiting its expression *in vivo*.

13. A pharmaceutical composition for inducing an apoptotic process in pathological cells comprising a pharmaceutically acceptable carrier and an active agent selected from the group consisting of:

(a) a fragment of the $TAF_{II}105$ polypeptide of SEQ. ID. NO: 2 or such a fragment modified by internal deletion, replacement or addition of one or more amino acids, wherein the fragment or the modified fragment has a dominant negative effect on the normal biological activity of the $TAF_{II}105$ polypeptide;

(b) an inhibitor or antagonist of the $TAF_{II}105$ polypeptide of SEQ. ID. NO: 2;

(c) a DNA sequence encoding the fragment or modified fragment of (a); and

(d) a DNA sequence directing expression of an antisense RNA sequence to SEQ.ID. NO.: 1, or a part thereof, and which is capable of inhibiting its expression *in vivo*.

14. A pharmaceutical composition according to Claim 13 wherein the active agent (a) is a polypeptide as defined in any one of Claims 7-11.

15. A pharmaceutical composition according to Claim 13 wherein the active agent (c) is a DNA molecule as defined in any one of Claims 1-5.

16. A pharmaceutical composition according to any one of Claims 13-15 for the treatment of cancer.

17. A method for promotion of apoptosis comprising administering to a subject in need, a pharmaceutical composition according to any one of Claims 13-16.

18. A pharmaceutical composition for treatment of pathological apoptosis of cells comprising a pharmaceutically acceptable carrier and an active agent selected from the group consisting of:

(e) a DNA molecule comprising the DNA sequence of SEQ. ID. NO: 1;

(f) a DNA molecule consisting of a DNA sequence encoding the $TAF_{II}105$ polypeptide of SEQ. ID. NO: 2;

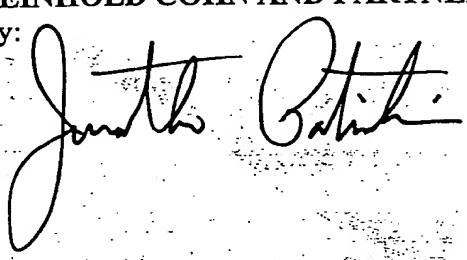
(g) a modified DNA sequence of (a) or (b) in which one or more nucleotide triplets have been added, deleted or replaced, wherein the polypeptide encoded by the modified DNA sequence retains the normal biological activity of the $TAF_{II}105$ polypeptide of SEQ. ID. NO: 2;

(h) the $TAF_{II}105$ polypeptide of SEQ. ID. NO: 2; and

(i) a modified polypeptide of (d) in which one or more amino acids have been added, deleted or replaced, wherein the modified polypeptide retains the normal biological activity of the $TAF_{II}105$ polypeptide encoded by SEQ. ID. NO: 2.

19. A pharmaceutical composition according to Claim 18 for the treatment of autoimmune diseases, inflammatory processes and viral or bacterial infections.

For the Applicants,
REINHOLD COHN AND PARTNERS
By:



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AACTCTGAATTGGTTGGCACACTCATTCAGTCATGTAAAGATGAACCATTTCT
TTTTATTGGAGCTCTACAAAAGAGAATCTTAGACATTGGTAAAAAGCATGAC
ATTACAGAACTTAACTCTGATGCTGTGAACTTGATCTCCCAAGCAACACAGG
AACGACTACGAGGCCTTCTAGAAAAACTGACTGCAATTGCTCAGCATCGAAT
GACTACTTACAAGGCAAGTGAAAATTACATCCTGTGTAGTGATACCAGGTCA
CAGCTCAAATTTCTTGAAAAGCTGGATCAATTGGAGAAGCAGAGAAAGGATT
TGGAAGAAAGAGAAATGTTACTTAAGGCAGCCAAGAGTCGTTCTAATAAAGA
AGATCCAGAACAGCTGAGATTAAAGCAGAAAGCCAAAGAGTTACAGCAATT
GGAACTTGCACAGATACAGCATAGAGACGCTAATCTCACAGCTCTTGCAGCT
ATTGGACCAAGGAAGAAGAGACCACTAGAATCTGGAATTGAGGGCTTAAAA
GACAACCTTCTTGCTTCTGGGACATCCAGCCTGACAGCCACCAAACAGTTGC
ATCGTCCAAGAATCACGAGAATCTGCCTCAGGGACTTGATATTTTGTATGGA
ACAGGAACGGGAGATGAAGTATTCTCGAGCTCTATACCTGGCCCTTCTG

Fig. 1

AAGTGACCACTCCACTCTTCCATCCACATCCTTGCTATTTACTGCCAAAGAAG
ACACAAAGCATTGTTGCACTGTCCTGAAATTTCAATTTCTGGAAAATAACACC
AACATGAAAGAGCATTGTTTACGATTAGAACTTTATTAACTCTTACCTAT

Fig. 1 (Con't)

GTLVTKVAPVSAPPKVSSGPRLPAPQIVAVKAPNTTTIQFPANLQLPPGTVLIKSNS
GPLMLVSPQQTVTRAETTSNITSRPAVPANPQTVKICTVPNSSSQLIKKVAVTPVK
KLAQIGTTVVTTVPKPSSVQSVAVPTSVVTVTPGKPLNTVTTLKPSSLGASSTPSN
EPNLKAENSAAVQINLSPTMLENVKKCKNFLAMLIKLACSGSQSPEMGQNVKKL
VEQLLDAKIEAEEFTRKLYVELKSSPQPHLVPFLKKSVVALRQLLPNSQSFIQQCV
QQTSSDMVIATCTTTVTTSPVVTTTVSSSQSEKSIIVSGATAPRTVSVQTLNPLAGP
VGAKAGVVTLHSVGPTAATGGTTAGTGLLQTSKPLVTSVANTVTTVSLQPEKPV
VSGTAVTLSLPAVTFGETSGAAICLPSVKPVVSFCWDHICKPVIGTPVQIKLAQPG
PVLSQPAGIPTGSSSKQLFSLFHVVQQPSGGNEKQVTTISHSSTLTIQKCGQKTMP
VNTIIPTSQFPPASILKQITLPGNKILSLQASPTQKNRIKENVTSCFRDEDDINDVTS
MAGVNLNEENACILATNSELVGTLIQSCKDEPFLFIGALQKRILDIGKKHDITELNS
DAVNLISQATQERLRGLLEKLTAIAQHRMTTYKASENYILCSDTRSQLKFLEKLD
QLEKQRKDLEEREMLLKAAKSRSNKEDPEQLRLKQKAKELQQLELAQIQHRDAN
LTALAAIGPRKKRPLESGIEGLKDNLLASGTSSLTATKQLHRPRITRICLRDLIFCM
EQEREMKYSRALYLALLKZPLHSSIHILAIYCQRRHKALLHCPEISISGKZHQHERA
LFTIRTLLTLTY

Fig. 2

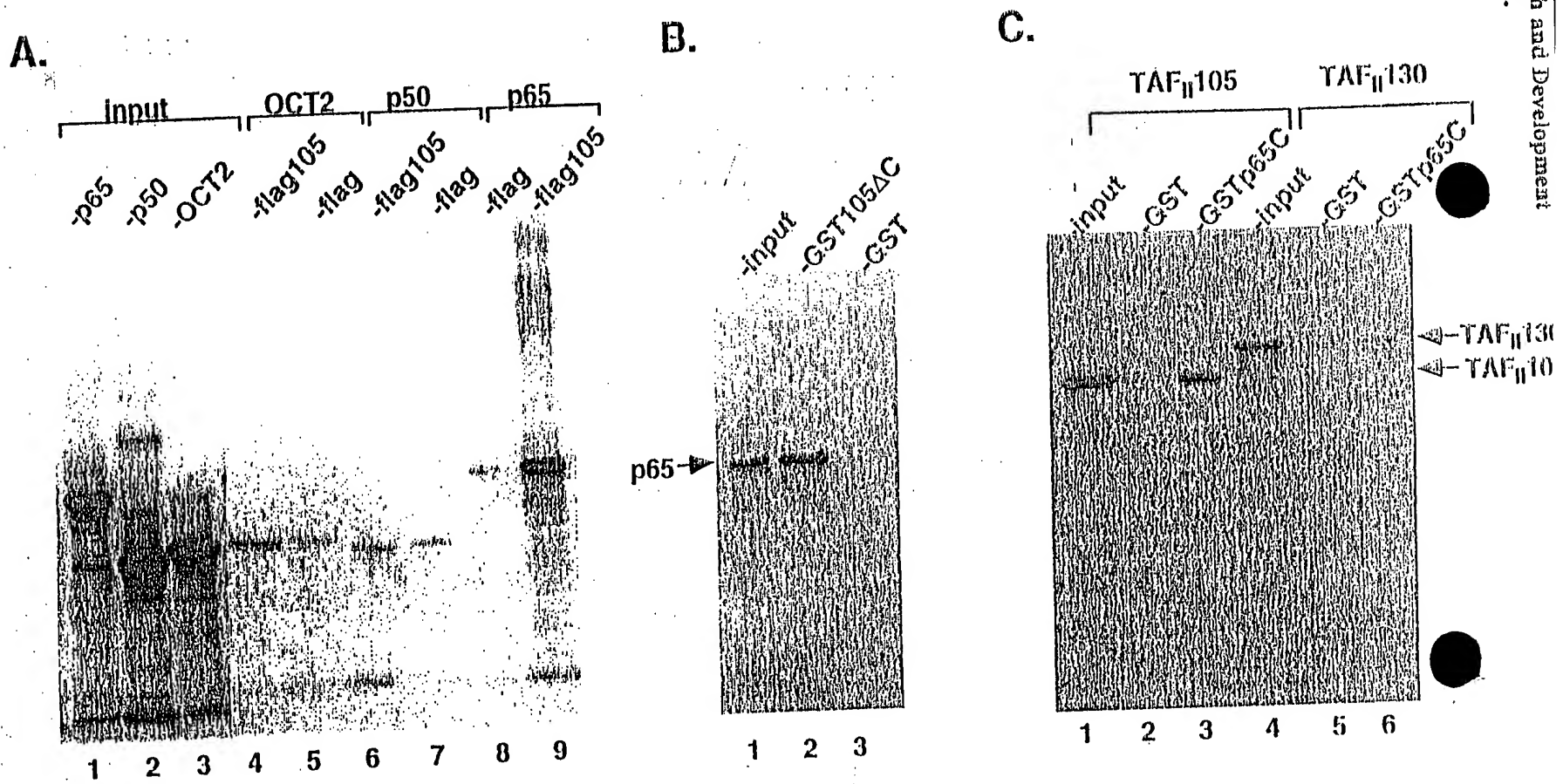


Fig. 3

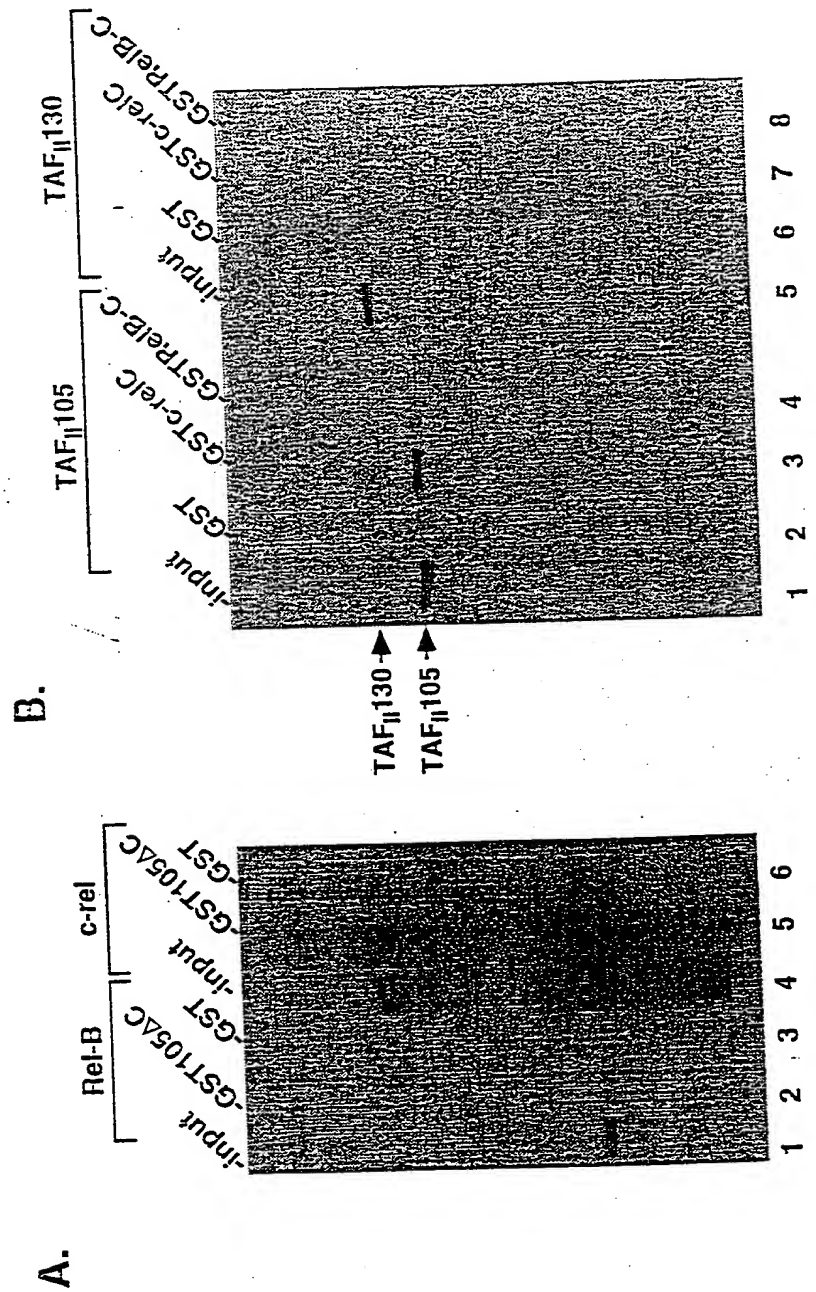

B.
TAFII130
TAFII105
GST-TRelB-C
GST-c-relC
GST
input
GST-TRelB-C
GST-c-relC
GST
input
1 2 3 4 5 6 7 8
A.
Rel-B
c-rel
GST-105AC
input
GST-105AC
GST
input
GST-105AC
GST
1 2 3 4 5 6

Fig. 4

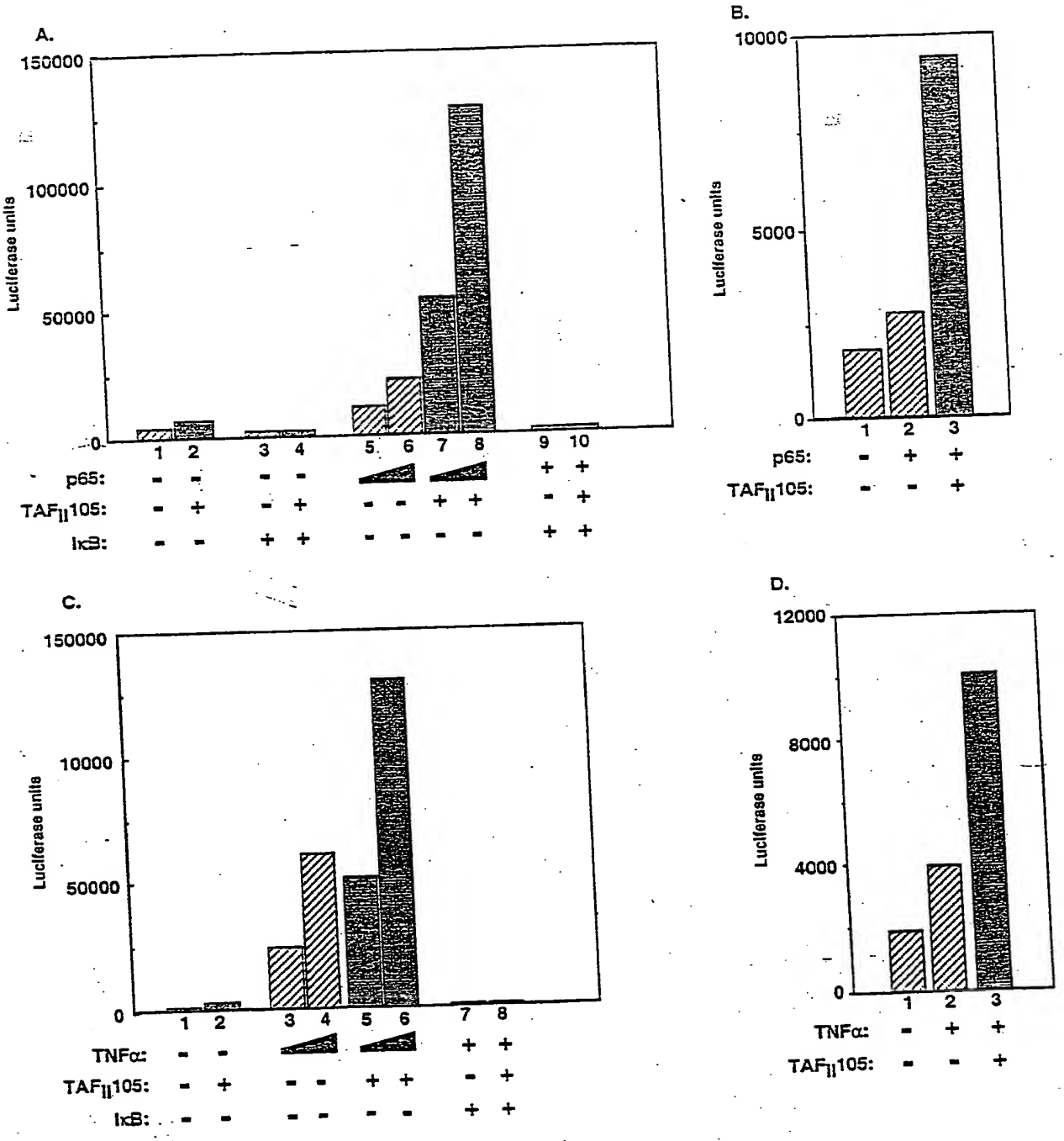

A.
Luciferase units
150000
100000
50000
0
1 2 3 4 5 6 7 8 9 10
p65: - - - - + + + + + +
TAFII105: - + - + - - + + - +
IκB: - - + + - - - - + +
B.
Luciferase units
10000
5000
0
1 2 3
p65: - + +
TAFII105: - - +
C.
Luciferase units
150000
10000
50000
0
1 2 3 4 5 6 7 8
TNFα: - - + + + +
TAFII105: - + - - + + - +
IκB: - - - - - - + +
D.
Luciferase units
12000
8000
4000
0
1 2 3
TNFα: - + +
TAFII105: - - +

Fig. 5

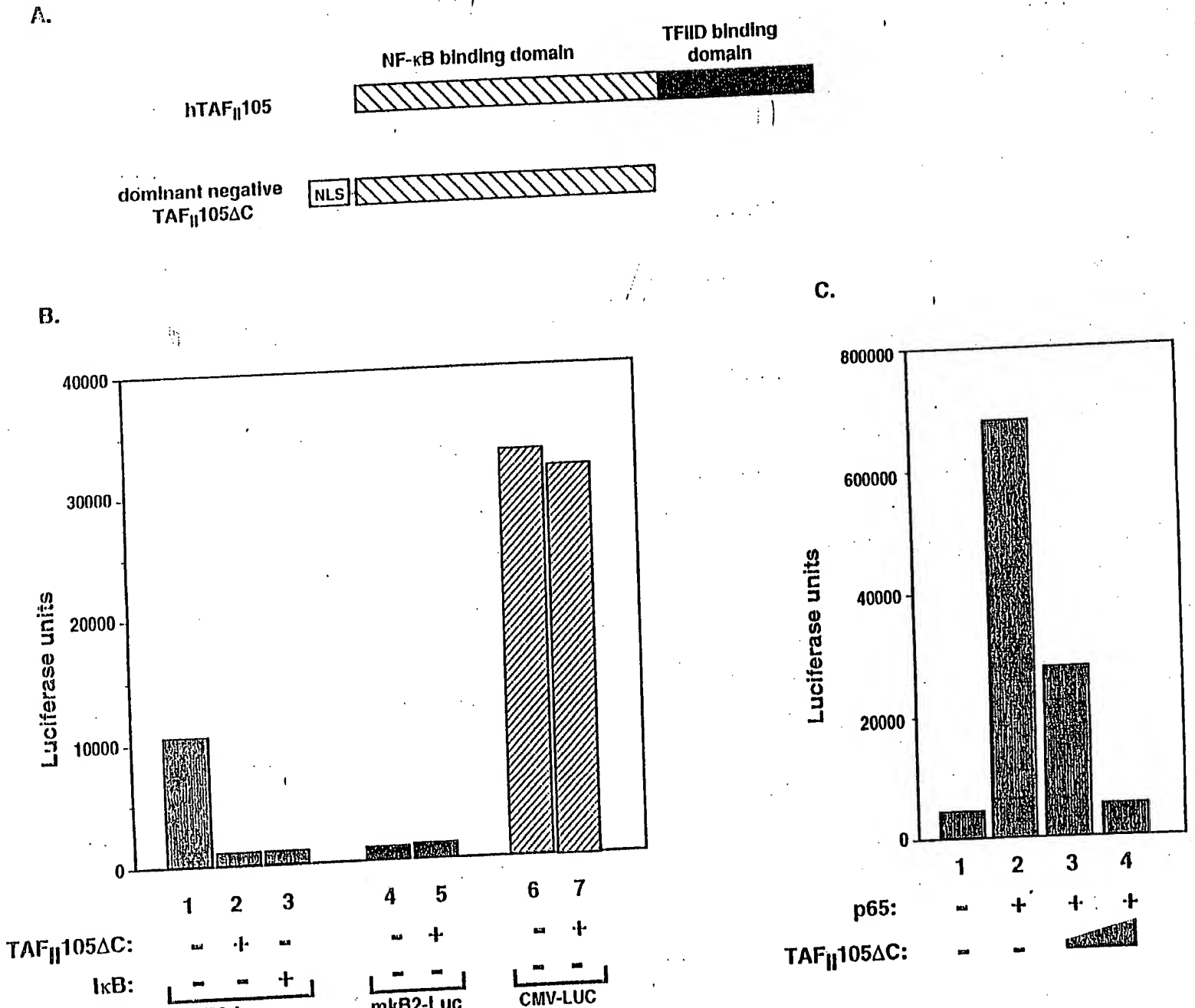
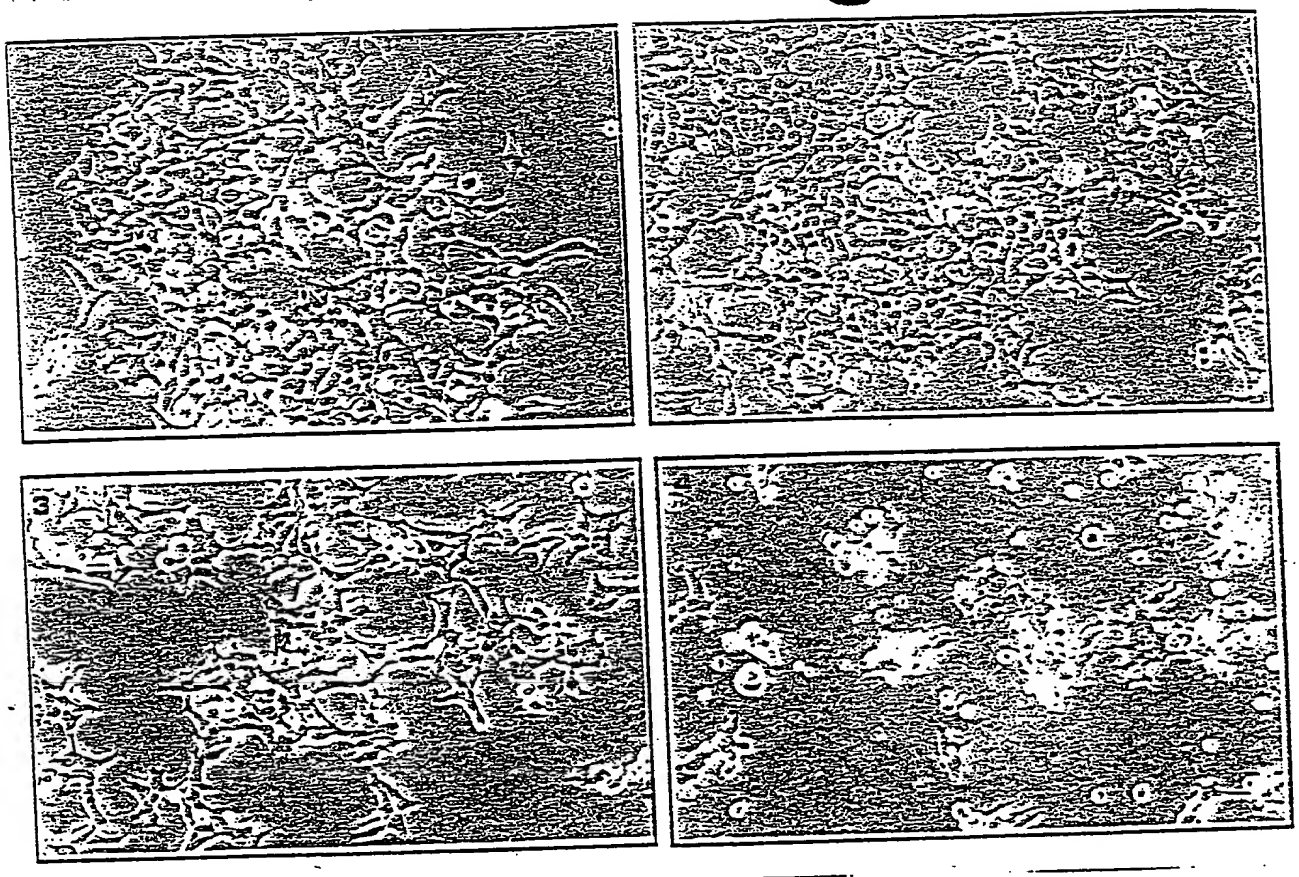
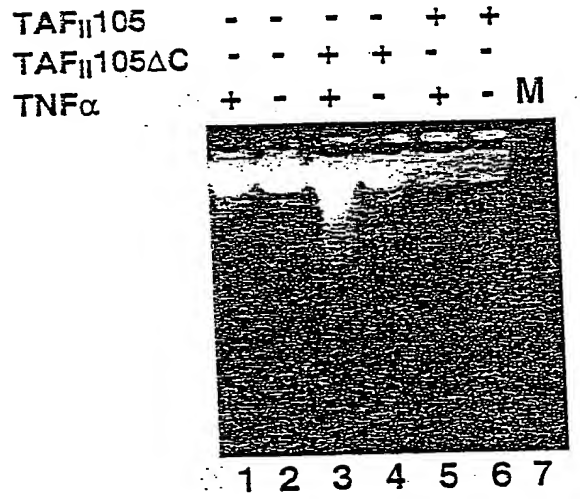


Fig. 6

A.


3

B.


TAFII105 - - - - + +
TAFII105ΔC - - + + - -
TNFα + - + - + - M
1 2 3 4 5 6 7

C.

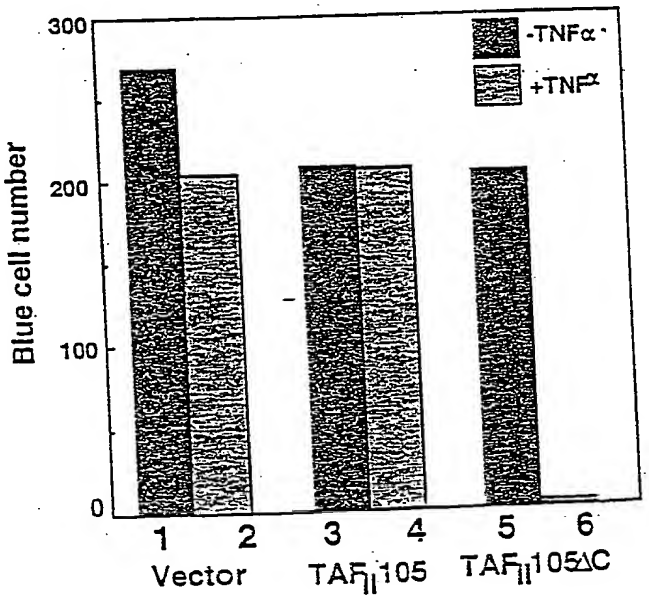
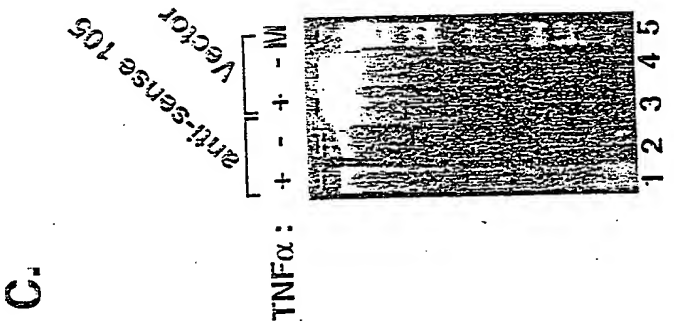
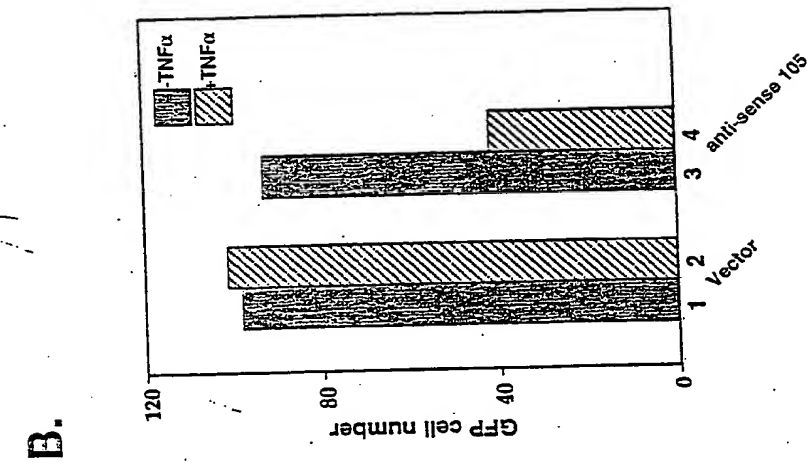

300
200
100
0
Blue cell number
-TNFα
+TNFα
1 2 3 4 5 6
Vector
TAFII105
TAFII105ΔC

Fig. 7


C.
TNFα :
anti-sense 105
Vector
M - - + + M
1 2 3 4 5


B.
-TNFα
+TNFα
GFP cell number
120
80
40
0
1 2 Vector
3 4 anti-sense 105

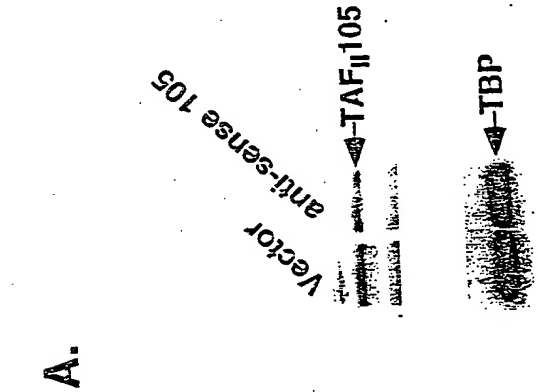
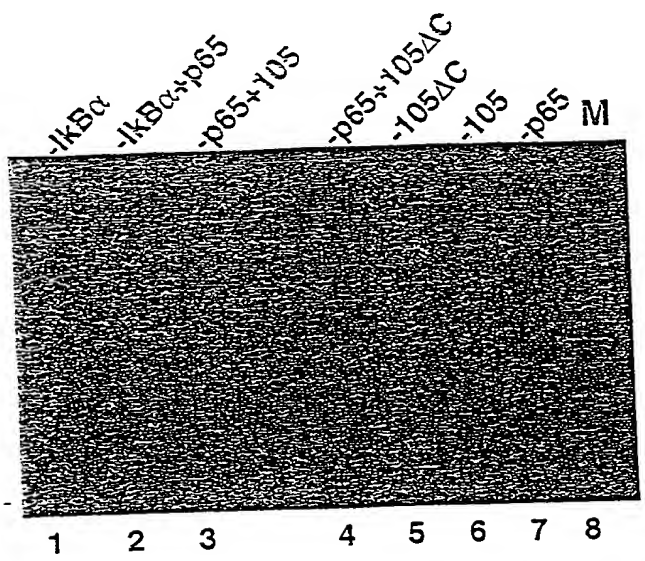
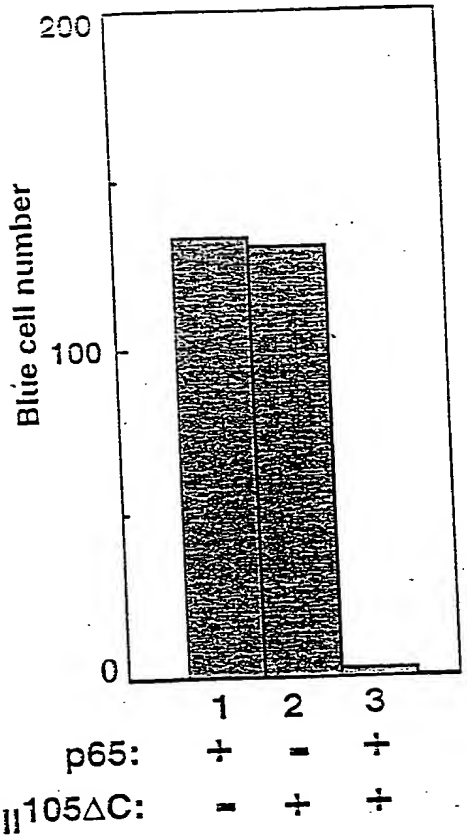

A.
Vector
anti-sense 105
TAFII105
TBP

Fig. 8

A.


-IκBα
-IκBα+p65
-p65+105
-p65+105ΔC
-105ΔC
-105
-p65
M
1 2 3 4 5 6 7 8

C.


200
100
0
Blue cell number
1 2 3
p65: + - +
TAF$_{II}$105ΔC: - + +

B.

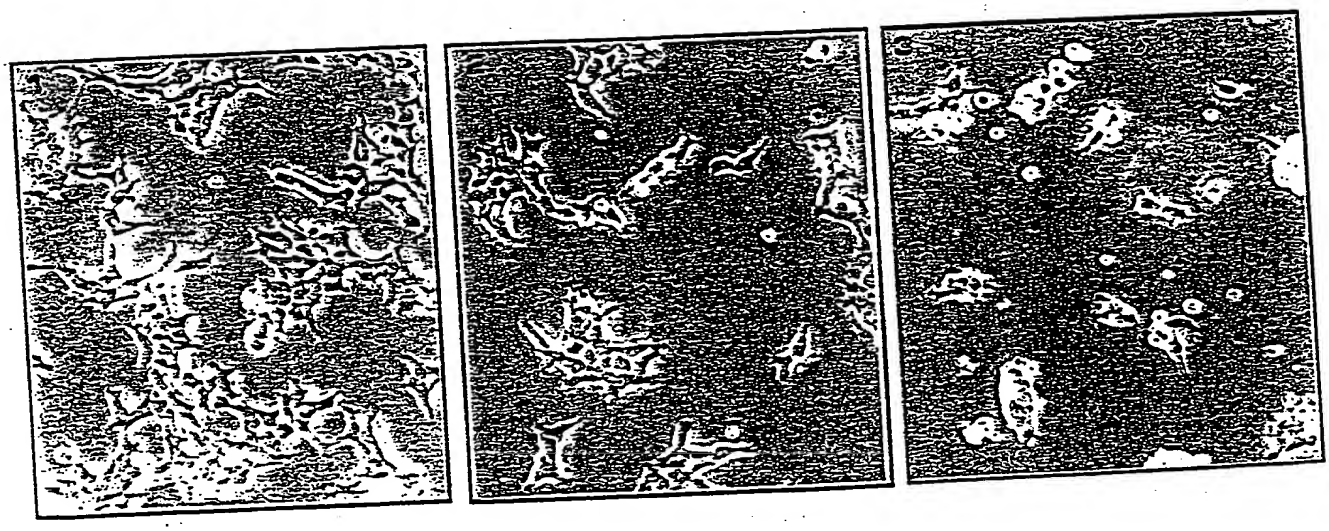


Fig. 9

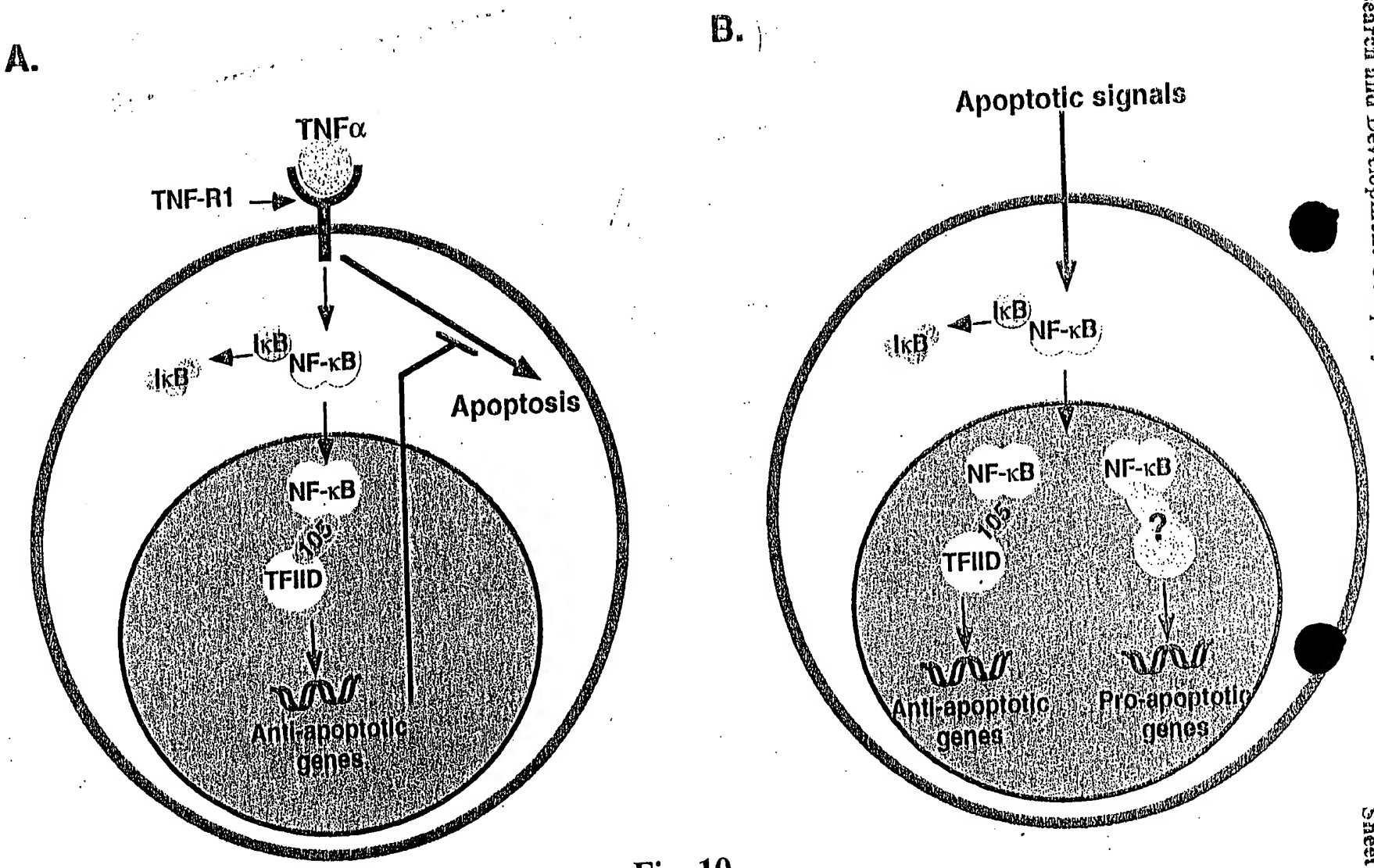


Fig. 10

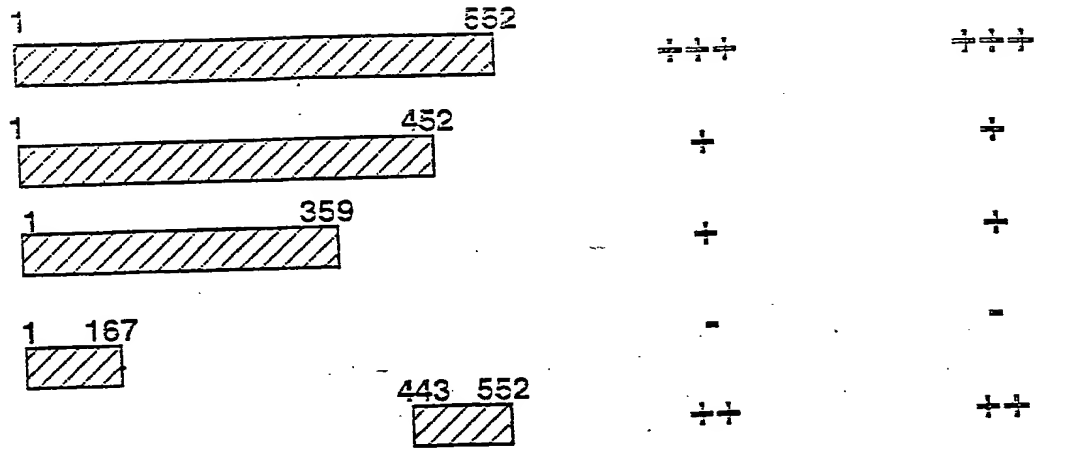
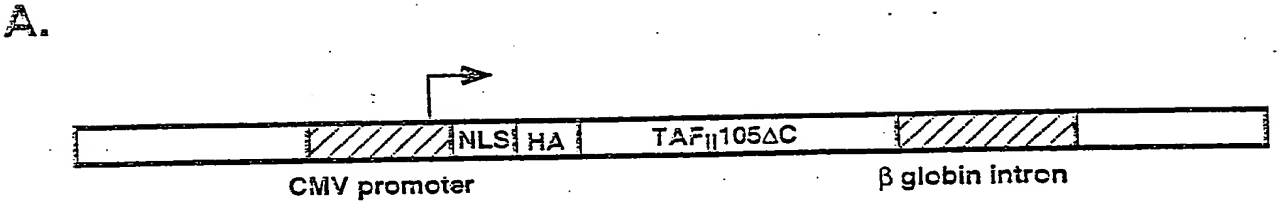

p65 binding
Cell death
1
552
+++
+++
1
452
+
+
1
359
+
+
1
167
-
-
443
552
++
++

Fig. 11


A.
NLS
HA
TAFII105ΔC
CMV promoter
β globin intron

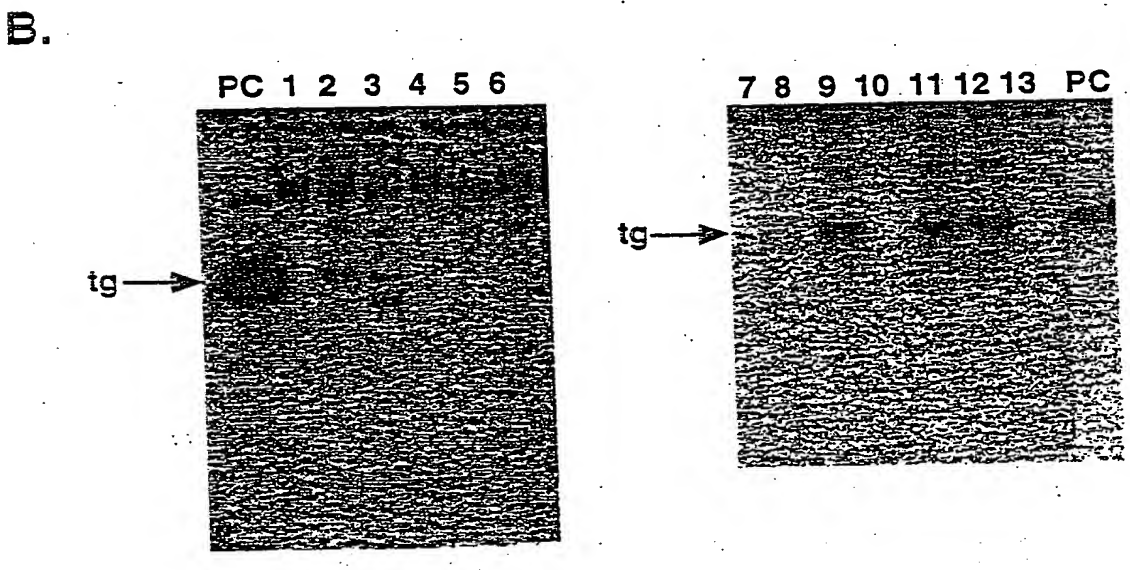

B.
PC 1 2 3 4 5 6
7 8 9 10 11 12 13 PC
tg
tg

Fig. 12

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